RESEARCH LETTER

Quorum-sensing signal production by *Agrobacterium vitis* strains and their tumor-inducing and tartrate-catabolic plasmids

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Received 4 November 2008; accepted 8 April 2009.
Final version published online 11 May 2009.

DOI:10.1111/j.1574-6968.2009.01627.x

Editor: Skorn Mongkolsuk

Keywords
acyl-homoserine lactone; crown gall; grapevine; opines; *Vitis*.

Abstract

Agrobacterium vitis strains, their tumor-inducing (pTi) and tartrate utilization (pTr) plasmid transconjugants and grapevine tumors were analyzed for the presence of *N*-acyl-homoserine lactones (AHLs). All wild-type *A. vitis* strains produced long-chain signals. PCR analysis of the *A. vitis* long-chain AHL synthase gene, *avsI*, showed the predicted amplicon. *Agrobacterium tumefaciens* UBAPF2 harboring various *A. vitis* pTi plasmids produced *N*-(3-oxo-octanoyl)-L-homoserine lactone encoded also by pTis of *A. tumefaciens*. UBAPF2 transconjugants carrying pTrs except for pTrTm4 and pTrAB3, also produced an AHL. UBAPF2 transconjugants carrying pTrAT6, pTrAB4 and pTrRr4 or pTiNi1 produced two additional AHLs not observed in the corresponding wild-type strains. We also provide evidence for *in situ* production of AHLs in grapevine crown gall tumors of greenhouse and field origin.

Introduction

Expression of certain bacterial genes frequently depends on the cell density of the bacterial population. This phenomenon, termed quorum-sensing (QS), is mediated by specific molecules called QS signals (White & Winans, 2008). Over 68 bacterial species contain at least one complete QS circuit. This includes the QS core proteins known as LuxI-type protein [the *N*-acyl-homoserine lactone (AHL) synthase], and a LuxR-type protein (the response regulator) (Case *et al*., 2008).

The specificity of AHLs is determined by the acyl side chain length, degree of its saturation and by the lack or presence of an oxo- or hydroxy-group in the C3 position (Beck von Bodman *et al*., 2003; Waters & Bassler, 2005). AHLs are characterized as long- or short-chain AHLs depending on whether their acyl moiety consist of more than eight or ≤ 8 carbons, respectively. QS plays a determinative role in the life of microbial communities as well as in several animal and plant diseases. Therefore, manipulating the signal production or degradation may provide a novel tool to control plant diseases (Cui & Harling, 2005). Details on the biochemical and molecular mechanism underlying QS regulation have recently been reviewed (Williams, 2007; White & Winans, 2008).

Crown gall disease on grapevine is most frequently caused by *Agrobacterium vitis*. Isolates of *A. vitis* are subclassified into octopine, nopaline and vitopine groups on the basis of the tumor-inducing (pTi) plasmid encoded opine utilization (by the bacterium) and synthesis (by the plant tumors) (Burr & Otten, 1999). Besides the pTi plasmid, *A. vitis* contains another plasmid called the tartrate utilization plasmid (pTr) (Szegedi *et al*., 1992, 1999; Otten *et al*., 1995). Like pTis, pTrs also show a high diversity both in their incompatibility properties and in the organization of TAR region (Crouzet & Otten, 1995; Otten *et al*., 1995; Szegedi & Otten, 1998). The ability of *A. vitis* to utilize tartrate as a carbon source contributes to the *Agrobacterium*-grapevine interaction (Kado, 1998; Salomone *et al*., 1998).

In the case of *Agrobacterium tumefaciens*, the QS system is closely associated with the pTi plasmid that harbors a single AHL synthase gene, *traI*. *TraI* is responsible for the synthesis of *N*-(3-oxo-octanoyl)-L-homoserine lactone (3-oxo-C8-HSL). This AHL signal, with the conjugal opines, is involved

in the regulation of conjugal transfer and plasmid replication of pTi (White & Winans, 2008). In previous studies with *A. vitis* strains, two strains were negative and one produced a weakly detectable AHL signal (Cha *et al.*, 1998). Tumorigenic *A. vitis* octopine-type strains AB3 and Tm4 produced short- and long-chain AHLs; in contrast, only long-chain AHLs were found to be produced by the vitopine-type strain S4 (Li *et al.*, 2005). The nontumorigenic *A. vitis* strain F2/5 produced six AHLs encoded both by a chromosomal-localized *luxI* ortholog, *avsI*, and by two putative *luxI* orthologs localized to the octopine-, and tartrate-catabolic plasmids (Li *et al.*, 2005). Three QS systems of F2/5, namely *aviR*, *avhR* and *avsI*/*avsR*, regulate the induction of hypersensitive response on tobacco and tissue necrosis on grapevine (Hao *et al.*, 2005; Hao & Burr, 2006).

To obtain an overview of AHL signals produced in *A. vitis*, we have screened tumorigenic grapevine isolates as well as their pTi and pTr plasmid transconjugants. We show that homologous sequences to *avsI* are present in the *A. vitis* strains and that field crown galls were found to contain detectable levels of AHLs.

Materials and methods

Bacterial strains and plasmids

Wild-type *A. vitis* strains, transconjugants in *A. tumefaciens* UBAPF2 containing *A. vitis* pTis and pTrs used in this work are listed in Supporting Information, Table S1. *Agrobacterium vitis* strains were grown on potato dextrose agar (PDA) at 28 °C medium before ethyl acetate extractions were prepared for AHL detection. *Escherichia coli* and *Chromobacterium violaceum* biosensor CV026 strains were grown in Luria–Bertani (LB) medium at 37 °C. *Agrobacterium tumefaciens* AHL-specific biosensor strain NTL4 (pZLR4) was grown in AB minimal medium (Chilton *et al.*, 1974) supplemented with 0.1% glucose and 0.01% yeast extract. All media were solidified with 16 g of Bacto Agar (Difco) per liter. For plasmid propagation and selection of transformations, media were supplemented with the following antibiotics: ampicillin (100 μg mL^{-1}), gentamicin (30 μg mL^{-1}), kanamycin (50 μg mL^{-1}), tetracycline (20 μg mL^{-1}) for *A. tumefaciens* and *E. coli*. For *A. tumefaciens* NTL4 (pZLR4), 5-bromo-4chloro-3-indolyl-β-D-galactopyranoside (X-gal) was used at 50 μg mL^{-1}.

Chemicals

Purified standards of AHLs were purchased from Quorum Sciences Inc., Coralville, IA, and Fluka, Switzerland. X-gal was purchased from Stratagene, La Jolla, CA. All other chemicals were purchased from Sigma Chemical Co., St. Louis, MO.

Signal extraction and detection

Bacterial culture supernatants resuspended from PDA plates were extracted with acidified ethyl acetate (aEtOAc) (1 mL of glacial acetic acid per 200 mL of ethyl acetate) for 30 min with shaking (150 r.p.m.) and centrifuged to separate the aqueous and ethyl acetate phases. The ethyl acetate phase was recovered and dried in a Savant Speed Vac. Twentyfold concentrated extracts were used in AHL assays.

AHLs were determined using five different reporter bacteria in which a detectable phenotype depends upon the LuxR-homolog's ability to sense exogenous AHLs (Table S2; McClean *et al.*, 1997; Winson *et al.*, 1998; Scott *et al.*, 2006). Extractions were screened using the *A. tumefaciens* NTL4 (pZLR4) (Table S2) in well-diffusion assays (Ravn *et al.*, 2001; Scott *et al.*, 2006) before performing thin-layer chromatography (TLC)-overlay detection assays (Shaw *et al.*, 1997; Farrand *et al.*, 2002). Violacein production by *C. violaceum* CV026 was tested on LB medium at 27 °C by 'T'-streak assays (McClean *et al.*, 1997). *Escherichia coli* JM109 strains with different AHL-receptor proteins were also used (Table S2; Winson *et al.*, 1998). Assay were carried out as described (Scott *et al.*, 2006). Mean values of the specific luminescence units were obtained with three independent samples. Each experiment was repeated at least twice.

TLC assays

Reverse-phase (RP)-TLC plates were used to determine AHL signal profiles. Concentrated aEtOAc extracts were spotted on to the TLC origin in 2-μL volumes and from 0.5- to 2-mL supernatant equivalents were loaded per lane onto a C18 RP-TLC plate (EMD Chemicals Inc., Gibbstown, NJ). Plates were developed in a 60% methanol : water mobile phase, dried and AHLs were detected as described (Scott *et al.*, 2006). AHL signals were identified with appropriate reference compounds. This involves determining and comparing retardation factors (Rf) of unknown samples to AHL reference compounds (McClean *et al.*, 1997; Shaw *et al.*, 1997). TLC analyses were repeated at least twice.

PCR analysis

DNA was prepared from overnight bacterial cultures using the Triton/sodium-azide lysis method (Abolmaaty *et al.*, 2000). Primers avsI-F2 (5′-AGCCGACATAAGCAGAC GCAACAG-3′) and avsI-R2 (5′-CGAAACATCCGCT CCAAAAACAC-3′) designed on the basis of *A. vitis* S4 sequence data (Hao & Burr, 2006) were used for experiments. These oligos yield a 447-bp amplification product. Reactions and detection were carried out as described (Hao & Burr, 2006).

Signal detection from greenhouse grown and natural crown gall tumors

Stems of *Vitis vinifera* cv. 'Sultanina' were inoculated by 48-h-old cultures of wild-type *Agrobacterium* strains (*c.* 5 μL of 10^8 cells mL^{-1} suspension). Plants were kept in the greenhouse under natural light. Tumors were harvested after 3 months for AHL extraction. Field tumors were collected from naturally infected plants in July in Hungary. After mechanical homogenization, tumors were extracted with four volumes of distilled water. Samples were centrifuged and the supernatants were extracted with one volume of aEtOAc followed by centrifugation to separate the phases. The ethyl acetate fractions were dried under nitrogen stream at room temperature and samples were redissolved in 1/20 volume of ethyl acetate.

Statistical analyses

Data were statistically analyzed using SAS version 9.1 (SAS Institute Inc., 2004, Cary, NC).

Results

Screening of wild-type *A. vitis* and *A. tumefaciens* transconjugates containing pTis and pTrs for AHL

We first screened wild-type *A. vitis* strains and *A. tumefaciens* UBAPF2 transconjugates with biosensor strain *C. violaceum* CV026. No response in violacein production was observed with the CviR-dependent AHL biosensor (data not shown). Next, aEtOAc extracts were prepared and screened by well-diffusion assay using the broad-spectrum AHL TraR-dependent *A. tumefaciens* NTL4 (pZLR4) biosensor. This assay showed the TraR-dependent production of β-galactosidase by all wild-type *A. vitis* strains and by all *A. tumefaciens* UBAPF2 transconjugants except pTrTm4 and pTrAB3 (data not shown). NTL4 (pZLR4) responds preferentially to 3-oxo-C8-HSL and to unsubstituted and 3-oxo signals, except for C4-HSL (Steindler & Venturi, 2007).

To determine the AHL species produced by wild-type *A. vitis* and their pTis and pTrs, our next screens involved the use of three *E. coli*-based AHL bioluminescence biosensor strains: JM109 (pSB401), JM109 (pSB536) and JM109 (pSB1075) (Table S2). No significant induction of light production was observed with extracts from wild-type *A. vitis* with short-chain JM109 (pSB401) and JM109 (pSB536) biosensors (data not shown). However, extracts from wild-type octopine-, nopaline- and vitopine-type *A. vitis* strains induced light production to significant levels in the long-chain LasR-dependent reporter JM109 (pSB1075) (Table 1).

To dissect AHL production encoded by a chromosomally localized synthase to those encoded by plasmids *A. tumefaciens* UBAPF2, carrying various *A. vitis*-derived pTis and pTrs (Table S1) were tested. AHL production in UBAPF2 transconjugants harboring pTiNi1 and pTiSz1 were compared with their wild-type strains using long-chain biosensor JM109 (pSB1075). This showed that the wild-type strain Sz1 is responsible for the activation of bioluminescence via the LasR receptor, and interestingly the introduction of pTiNi1 into plasmidless recipient strain UBAPF2 activated bioluminescence production to levels significantly greater than that in the wild-type strain Ni1 (Fig. 1). The reason(s) for this observation is not completely accounted for in this work.

AHL profiles of wild-type *A. vitis* strains

The seven vitopine-type strains assayed contained two signals, a short- and long-chain AHL detectable by the TraR-dependent reporter. An AHL standard derived from *A. tumefaciens* NT-1(pTiC58Δ*accR*), which overproduces 3-oxo-C8-HSL via TraI (Beck von Bodman *et al.*, 1992), gave an Rf value of *c.* 0.53, which was identical to Rf values of the short-chain AHL in the seven vitopine-type strains tested (Fig. 2a, lane 9 compared with lanes 2–8). The long-chain AHL signal(s) detected from vitopine-type strains remained near the origin, gave an Rf value of *c.* 0.06, which is consistent with long-chain AHL(s) detected with extracts from the nontumorigenic *A. vitis* strain F2/5 (Li *et al.*, 2005). The long-chain AHL spot detected from F2/5 appears to be a mix of different AHLs encoded by the *avsI* locus that vary in oxo-substitution on third carbon and in saturation of the acyl-side chain (Li *et al.*, 2005). Octopine- and nopaline-type strains also produce a detectable long-chain AHL(s) (Fig. 2b). The octopine-type strains AB3, AT6 and Tm4 as well as the nopaline-type strains AB4, Ni1 and Rr4 produced

Table 1. Bioluminescence activation in long-chain AHL reporter JM109 (pSB1075) to extracts from wild-type *Agrobacterium vitis* strains

Opine type or controls	Strains	Bioluminescence*
Octopine	AB3	3020 ± 262
	AT6	5132 ± 314
	Tm4	2842 ± 76
Nopaline	AB4	3660 ± 96
	Ni1	713 ± 145
	Rr4	3595 ± 550
Vitopine	NW221	2510 ± 53
	RF2/1	3200 ± 193
	S4	3639 ± 185
	SF93	2367 ± 667
	Sz1	1130 ± 251
	T1/7	356 ± 36
Controls	aEtOAc	132 ± 4
	3-oxo-C12-HSL†; 10 nM	3337 ± 405

*Bioluminescence in relative light units.

†3-oxo-C12-HSL, *N*-3-oxododecanoyl-homoserine lactone.

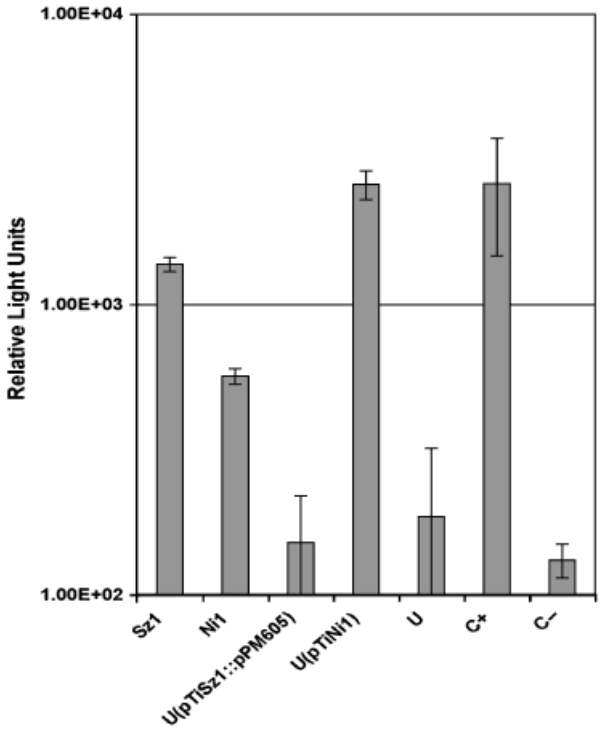

Fig. 1. Detection of long-chain AHLs in extracts of *Agrobacterium vitis* strains and UBAPF2 transconjugants carrying a tumor-inducing plasmid. Abbreviations include *A. vitis* wild-type strains Sz1 and Ni1; UBAPF2 carrying pTiSz1::pPM605 or pTiNi1. U, plasmidless *A. tumefaciens* recipient UBAPF2; C+, positive control of pure 3-oxo-C12-HSL (10 nM); C−, negative control of aEtOAc. All bioluminescent measurements are with long-chain AHL-dependent *Escherichia coli* biosensor strain JM109 (pSB1075).

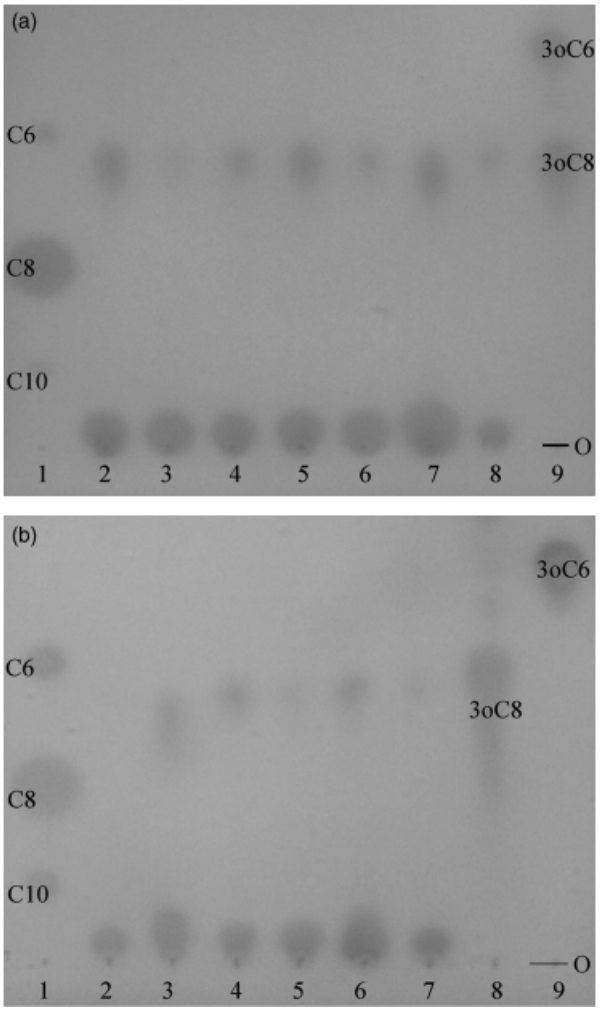

Fig. 2. Detection of *N*-acyl-homoserine lactones (AHLs) from extracts of *Agrobacterium vitis* strains. (a) Detection of AHLs from extracts of vitopine-type strains. Lanes: 1, AHL standards of C6, C8 and C10; 2, *A. vitis* C6/1; 3, *A. vitis* NW221; 4, *A. vitis* RF2/1; 5, *A. vitis* S4; 6, *A. vitis* SF93; 7, *A. vitis* Sz1; 8, *A. vitis* T1/7; 9, 5-μL extract from *Agrobacterium tumefaciens* NT1 (pTiC58Δ*accR*) which overproduces 3-oxo-C8-HSL (3oC8) and pure 3-oxo-C6-HSL (3oC6) (4 μL of 100 nM) as standards; O, position of extract origin. (b) Detection of AHLs from extracts of nopaline- and octopine-type strains of *A. vitis*. Lanes: 1, AHL standards of C6, C8 and C10; 2, *A. vitis* nopaline-type strains AB4; 3, Ni1; 4, Rr4; 5, octopine-type strains AB3; 6, AT6; 7, Tm4; 8, 4-μL extract from *A. tumefaciens* NT1 (pTiC58Δ*accR*), which overproduces 3oC8; 9, pure 3oC6 (4 μL of 100 nM) as standards; O, position of extract origin. Not shown in this image is identification of a signal that comigrates with 3oC8 for nopaline-type strain AB4 (lane 2), which was identified in repeated experiments with extracts of *A. vitis* AB4 (data not shown).

a second AHL that comigrated with the *Agrobacterium tumefaciens* pTiC58-encoded signal, 3-oxo-C8-HSL, with Rf values between 0.48 and 0.50 (Fig. 2b).

AHL profiles of tumor-inducing plasmid (pTi) transconjugants

AHL signal profiles of UBAPF2 transconjugants carrying octopine (three)-, nopaline (two)-, and vitopine (two)-type tumor-inducing plasmids of *A. vitis* showed the production of one AHL with Rf values consistent with the 3-oxo-C8-HSL (Fig. 3a and data not shown). 3-Oxo AHLs characteristically produce tailing spots with diffuse edges whereas the 3-unsubstituted and 3-OH forms produce circular spots with sharp edges (Shaw *et al.*, 1997). Consistent with AHL determinations using the long-chain LasR-dependent bioluminescent biosensor strain JM109 (pSB1075) (Fig. 1 and Table 1), extracts from transconjugant UBAPF2 (pTiNi1) showed, in addition to the 3-oxo-C8-HSL signal, a novel and abundant long-chain signal. This explains the LasR-dependent activation of bioluminescence shown in Fig. 1. This AHL signal exhibited migration and detection characteristics using NTL4 (pZLR4) consistent with an AHL that contains an acyl chain longer than 10 carbons and either containing a hydroxyl group substitution or unsubstituted at carbon number three of the acyl side chain (Fig. 3a). This presumed novel long-chain spot, putatively assigned 3-OH-

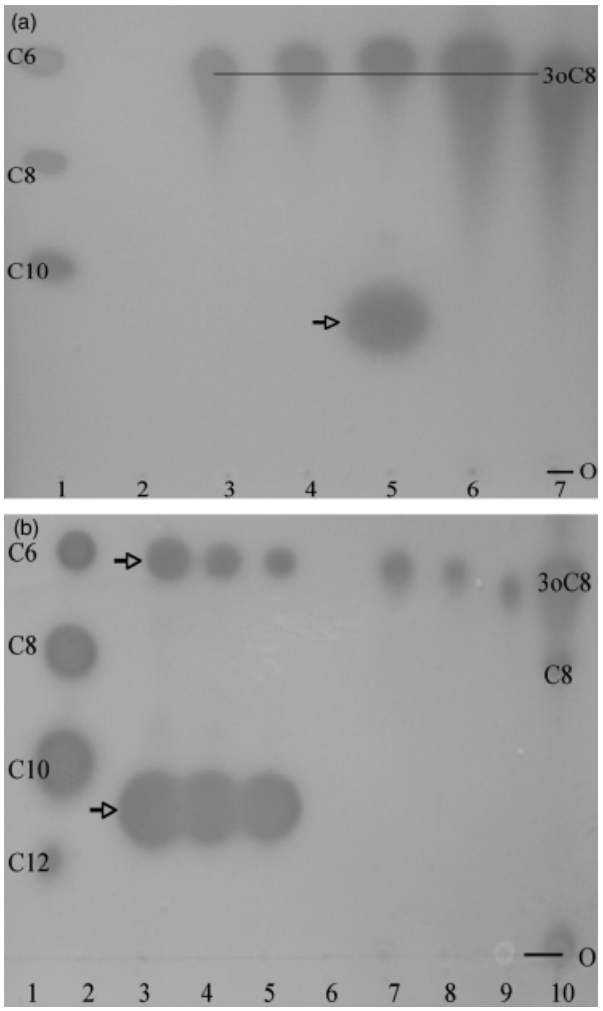

Fig. 3. Detection of autoinducers produced by tumor-inducing and tartrate-catabolic plasmids of *Agrobacterium vitis* strains. (a) Detection of acyl-HSL from extracts of transconjugants containing tumor-inducing plasmids of the octopine, nopaline and vitopine types from *A. vitis* strains. Lanes: 1, AHL standards, C6, C8 and C10; 2, *Agrobacterium tumefaciens* pTi plasmidless strain UBAPF2; 3, UBAPF2 transconjugants containing octopine-type tumor-inducing plasmid, pTiAB3; 4, pTiAT6; 5, UBAPF2 transconjugants containing nopaline-type plasmid, pTiNi1; 6, GV3101 transconjugant-containing octopine-type pTiTm4; 7, UBAPF2 transconjugant-containing vitopine-type plasmid pTiSz1; O, unexpected TraR-detected signal from transconjugant UBAPF2 containing nopaline-type Ti plasmid, pTiNi1 and position of the extract origin. (b) Detection of acyl-HSL from extracts of transconjugants containing tartrate-catabolic plasmids (pTr) from *A. vitis* strains. Lanes: 1, AHL standards fully reduced, C6, C8, C10 and C12; 2, *A. tumefaciens* UBAPF2; 3, UBAPF2 tartrate-catabolic plasmid transconjugants, pTrAT6; 4, pTrAB4; 5, pTrRr4; 6, pTrAB3; 7, pTrF2/5; 8, pTrS4; 9, pTrNW221; 10, *A. tumefaciens* strain NT1 (pTiC58ΔAccR) that over produces 3-oxo-C8-HSL (3oC8) and C8-HSL AHL signals. The production of C8-HSL (C8) and a detected signal at the origin in lane 10 is due to overloading. O, unexpected TraR-detected bioactive signals from UBAPF2 transconjugants containing pTrAT6, pTrAB4 or pTrRr4 (lanes 3, 4 and 5) noted in rectangle boxes and position of the origin.

C12-HSL, gave an Rf value of 0.25, whereas the short-chain spot gave an Rf value identical to the 3-oxo-C8-HSL standard of 0.53 (Fig. 3a and data not shown).

AHL profiles of tartrate-catabolic plasmids (pTr) transconjugants

Tartrate-catabolic plasmids harbored in UBAPF2 produced at least one AHL except for transconjugants carrying pTrAB3 and pTrTm4 (Fig. 3b and data not shown). They were grown in AB medium with tartrate as the sole carbon source to test if tartrate induces the production of AHLs. Their growth on tartrate did not induce AHL production (data not shown). Biosensor strain NTL4 (pZLR4) detected a single AHL from vitopine-type strain derived pTrS4 and pTrNW221 and octopine-type-strain derived pTrF2/5 (Li *et al.*, 2005), which exhibited Rf values and detection characteristics identical to 3-oxo-C8-HSL (Fig. 3b). UBAPF2 transconjugants harboring pTrAT6, pTrAB4 and pTrRr4 produced two novel AHL spots, which were different from those detected from their wild-type strains (Figs 3b and 2b). The novel long-chain signal spot gave an Rf of 0.24 whereas the novel short-chain signal spot gave an Rf of 0.61, which was close to the Rf value (Rf = 0.63) of the known standard, C6-HSL (Fig. 3b).

Identification of *avsI* amplicon in wild-type *A. vitis* strains

To determine if an *avsI* homolog, responsible for long-chain AHL production in *A. vitis* strain F2/5, is present in the *A. vitis* strains, *avsI*-specific primers were used with total DNA of various *A. vitis* strains in PCR assays. The predicted *avsI* homologous amplicon of 447 bp was detected in all octopine-, nopaline-, and vitopine-type strains analyzed (Fig. 4). This is consistent with the detection of a long-chain AHL spot(s) with biosensor NTL4 after TLC separation.

AHLs detected in natural field grapevine crown gall tumors

To assess if field or greenhouse-grown tumors contain detectable levels of AHLs, NTL4 (pZLR4) seeded in AB minimal medium were used in well-diffusion assays. AHLs were detected from the extracts of the five natural field tumors assayed while one of the two greenhouse-grown tumors also contained detectable AHLs (Fig. 5). Extracts from wild-type leaf tissues of *V. vinifera* cv. 'Sultanina' grape did not contain detectable concentrations of AHLs (Fig. 5). In total 21 naturally occurring octopine-, nopaline- and vitopine-type field crown galls of 'Riesling', 'Ezerfürtü' and 'Kunleány' varieties were assayed for the presence of signal molecules. Forty-two to 50% of these tumor extracts showed

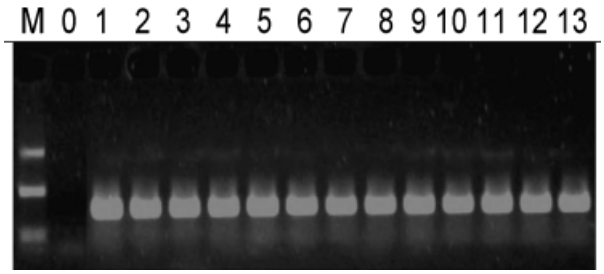

Fig. 4. PCR analysis of *Agrobacterium vitis* DNAs with avsI-F2/avsI-R2 specific primers. All tested strains of *A. vitis* produced the expected amplicon of the *avsI* gene region. M, size markers (1794, 753 and 191 bp), 0, DNA-free control. Lanes 1–13 are *A. vitis* AT6, Tm4, AB3, Zw2, AT1, AB4, Ni1, Rr4, S4, Sz1, NW221, SF93 and F2/5 in the same order. *Agrobacterium vitis* S4 (lane 9) and F2/5 (lane 13) strains were used as controls (Hao & Burr, 2006).

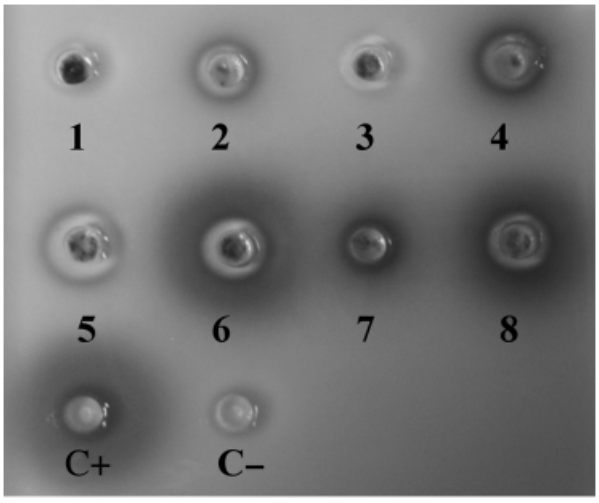

Fig. 5. Detection of AHLs from greenhouse-grown and field grapevine crown galls by diffusion well assay with biosensor strain NTL4 (pZLR4). 1, aEtOAc extracts from wild-type *Vitis vinifera* cv. 'Sultanina' leaf tissue; 2, 'Sultanina' greenhouse tumor induced by *Agrobacterium tumefaciens* strain Ag12 that carries an octopine-type *Agrobacterium vitis* Ti plasmid; 3, 'Sultanina' greenhouse tumor induced by *A. vitis* AB3; 4, *V. vinifera* cv. 'Teréz' field tumor; 5–6, *V. vinifera* 'R-73' field tumors; 7–8, *V. vinifera* 'R-68' field tumors. Standards include C+, 10 pmol of 3-oxo-C6-HSL in aEtOAc and C−, aEtOAc solvent only.

detectable levels of AHLs using the TraR-dependent biosensor strain (data not shown).

Discussion

This work shows that strains of *A. vitis* produce 3-oxo-C8-HSL and at least one long-chain QS AHL-type signal. Wild-type *A. vitis* strains contain sequences homologous to the *avsI* gene responsible for the synthesis of the long-chain AHLs. We also show that AHL signals can be detected from *A. tumefaciens* transconjugants harboring different *A. vitis* tumor-inducing (pTi) and tartrate-catabolic (pTr) plasmids. Thus QS systems may be operational on transmissible plasmids of *A. vitis*. *Agrobacterium tumefaciens* UBAPF2 strains containing pTrTm4 or pTrAB3, failed to produce any detectable AHL signal. UBAPF2 transconjugants harboring *A. vitis* pTrAB4, pTrRr4 and pTrAT6 or tumor-inducing plasmid pTiNi1 produce one or two novel signals that were not observed in AHL-profiles of the corresponding wild-type *A. vitis* strains. More significantly, TraR-dependent AHL signals were found in extracts of naturally occurring and greenhouse-grown grapevine crown gall tumors. Furthermore, one or more long-chain AHLs appear to be characteristic of the *A. vitis* species.

Agrobacterium tumefaciens produces a single pTi-encoded AHL, 3-oxo-C8-HSL, which along with a corresponding opine produced from the resulting crown gall tumor induces the conjugal transfer and increase the copy number of Ti plasmid (Li & Farrand, 2000; Farrand *et al.*, 2002; White & Winans, 2008); therefore, AHL production indirectly contributes to the virulence of *A. tumefaciens*. *Agrobacterium vitis* has more complex QS regulation systems. The non-tumorigenic *A. vitis* strain F2/5 harbors both chromosomal- and plasmid-encoded *luxI/luxR* type genes coding for at least six AHLs (Li *et al.*, 2005), although little is known on the AHL production of various tumorigenic *A. vitis* isolates. To assess the QS systems in octopine-, nopaline- and vitopine-type *A. vitis*, we have studied a set of tumorigenic *A. vitis* strains. All of the tested pathogenic *A. vitis* strains produced AHLs that were detectable by the *A. tumefaciens* NTL4 (pZLR4) biosensor strain. All of the 15 pTi or pTr plasmid transconjugants derived from *A. vitis* octopine-, nopaline- and vitopine-type strains, except for pTrTm4 and pTrAB3 produced AHLs indicating that most plasmid types contain a QS regulation system.

The *luxI* ortholog in *Rhodobacter capsulatus*, *gtaI*, is responsible for the synthesis of a major long-chain AHL signal and transfer of *gtaI* to *E. coli* is an example of a *luxI*-homolog-containing recombinant *E. coli* that does not produce the native AHL (Schaefer *et al.*, 2002). In contrast, the transfer of the *Yersinia pestis yspI* into *E. coli* strain BL21 showed similar RP-TLC AHL profiles to the wild-type *Y. pestis* strain and this was confirmed using mass spectrometric analysis (Kirwan *et al.*, 2006). In contrast, the expression of *avsI* gene from *A. vitis* biocontrol strain E26 in *E. coli* led to the production of a subset of the AHLs produced by the wild-type strain, including a long-chain and a short-chain AHL not previously identified (Wang *et al.*, 2008). The novel TraR-detectable putative AHLs observed in this work with transconjugants of plasmidless *A. tumefaciens* UBAPF2 harboring *A. vitis* tartrate-catabolic plasmids pTrAB4, pTrRr4, pTrAT6 or tumor-inducing plasmid pTiNi1 may be due to the intrinsic selectivity of the AHL synthase for a particular subset of a pool of available acyl-ACP substrates available in the *A. tumefaciens* UBAPF2

surrogate host, which may differ from the available acyl-ACP substrates in wild-type *A. vitis* strains. This difference may be responsible for the novel AHLs detected in the UBAPF2 transconjugants in this work.

This work shows the detection of potentially bioactive AHLs from tumor extracts of both field- and greenhouse-grown grapevine plants. Our work shows that QS signals are present in the grapevine tumors. It will be interesting to determine the identity of the AHL signals present in field grapevine tumors, the non-agrobacterial members of the grapevine tumorsphere consortium, and to determine if the non-*Agrobacterium* species contribute to the AHL signals detected from tumors.

To our knowledge, this is the first systematic survey of AHL production by octopine-, nopaline- and vitopine-type *A. vitis* strains and their tartrate-utilizing and tumor-inducing plasmids. We show that all wild-type *A. vitis* strains produce a characteristic long-chain signal(s) and short-chain signal(s) produced by various transmissible tumor-inducing and tartrate-catabolic plasmids derived from wild-type *A. vitis* strains in the surrogate *A. tumefaciens* host strain UBAPF2. In addition, we provide evidence of a locus that directs the production of long-chain AHLs in octopine-, nopaline- and vitopine-type *A. vitis* strains using primers specific for a *luxI* homolog, *avsI*, previously characterized in nontumorigenic *A. vitis* strain F2/5 (Hao & Burr, 2006). Our data also suggest that AHLs are being produced *in situ* within infected grapevine crown gall tumors.

Acknowledgements

Authors are grateful to Ms Erzsébet Puskás for helpful technical assistance, to Professor Léon Otten (IBMP, Strasbourg, France) for bacterial strains. We thank Stephen K. Farrand, Department of Microbiology, University of Illinois at Urbana-Champaign, Urbana, IL, for providing bacterial biosensor strain NTL4 (pZLR4). This work was supported by the USDA NRI Competitive Grants Program/USDA award number 2002-35319-12577, Dean's Summer Research Fellowship Grants from the College of Science, Rochester Institute of Technology (M.A.S.) and by the OTKA (Hungarian Scientific Research Foundation) grant no. K-68053 (E.S.). It was also partially funded by NRI Competitive Grants Program/USDA award number 2006-35319-16558 and NSF Microbial Geneome Sequencing Program SA6624 (T.J.B.). Undergraduate students (N.L. and R.S.) were supported by Summer Undergraduate Research Award from the College of Science, Rochester Institute of Technology, Rochester, NY.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Wild-type *Agrobacterium* strains.
Table S2. AHL signal biosensor strains.

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